Leucine in the Sky with Diamonds

New structures of LD motifs bound to the focal adhesion targeting domain show that the motifs are recognized as amphipathic α helices. The structures highlight the structural and functional diversity of leucine and aspartic acid-based targeting motifs.

Leucine-rich sequences tinged with aspartic acid residues give rise to several remarkably diverse signaling motifs. Acidic cluster-dileucine sequences of the form (D/E)XXXLL target certain receptors for endocytosis through adaptor protein (AP) complexes (Robinson and Bonifacino, 2001). The seemingly similar acidic cluster-dileucine sequence DXXLL targets another set of sorting receptors for endosomal trafficking between the Golgi apparatus and lysosomes (Misra et al., 2002; Shiba et al., 2002). The DXXLL motif, however, is recognized by a completely different set of adaptors from the (D/E)XXXLL signal. A third sequence motif of the form LDXLLXXL is known as the LD motif and occurs in proteins of the paxillin superfamily (Tumbarello et al., 2002). Paxillin is a scaffolding adaptor that brings together structural and signaling proteins at focal adhesions. The primary function of LD motifs is to interact with four helix bundles known as the focal adhesion targeting (FAT) domain. Arold and coworkers have now determined the structures of two different LD motifs from paxillin bound to the FAT domain of focal adhesion kinase (FAK) and show how the functional diversity of Leu- and Asp-based signals is mirrored by their structural diversity (Hoellerer et al., 2003).

Modular protein and lipid binding domains, of which the FAT domain is an example, are the fundamental building blocks of eukaryotic signaling proteins (Pawson and Nash, 2003). A great many of these domains recognize short linear amino acid sequence motifs. By now, most of the known examples have been characterized structurally. SH2, PTB, PDZ, FHA, FERM, VHS, and GAE domains and 14-3-3 proteins all bind short sequence motifs (Pawson and Nash, 2003). The structures of all of these domains and proteins have been determined in complexes with bound peptides and show that the peptides bind in extended β or β-like conformations. The β conformation is advantageous since it allows for the maximum exposure of binding determinants within a short motif to the surface of a compact domain. SH3 and WW domains bind peptides that adopt type II polyproline (PPII) helices, another relatively extended conformation.

There are far fewer cases of signaling proteins that bind to α-helical recognition motifs. Calmodulin, which wraps around helical CaM binding domains in its Ca^{2+}-activated state, is probably the best known example in this category (Crivici and Ikura, 1995). The new structures show that the LD motif peptides form amphipathic helices, as had been predicted from sequence gazing.

The LD motif/FAT domain pair adds to the select category of α-helical signaling motif/domain combinations. It makes a sharp contrast to the binding of the acidic cluster dileucine motif to the VHS domain in an extended conformation, highlighting the structural diversity of Asp- and Leu-containing signaling motifs.

As with most other pairings between domains and motifs, not every LD motif binds to every FAT domain. Of paxillin's five LD motifs, only the second and fourth bind to the FAK FAT domain, whereas the first, second, and fourth bind to vinculin. The third and fifth motifs have no known ligands. Interactions between the FAT domain and residues immediately N-terminal to the first Leu appear to account for these differences. Motifs 2 and 4 contain a Glu at the −1 position (where the first Leu is numbered 0) that interacts with a basic residue on the FAK FAT domain, and is missing in the other LD motifs.

Why do some proteins, such as paxillin, contain so many LD motifs? One explanation might be that each LD motif recruits one FAT domain protein, thereby scaffolding a large assembly. Another one, not necessarily exclusive of the first, might be that more than one motif can bind simultaneously to a single FAT domain, thereby enhancing affinity. Arold's study provides structural support for the second idea, although this remains to be fully corroborated by measurements of binding stoichiometry and in vivo analysis of site function. Two independent binding sites for LD motifs are present on the surface of the FAT domain. One site is formed by helices 1 and 4, the other by helices 2 and 3. Both sites have a similar gently concave shape and a hydrophobic character, along with one key basic residue poised to interact with the Asp of the motif. Indeed, intact paxillin binds to the FAK FAT domain 10-fold more tightly than an individual LD motif, supporting the idea that multiple LD motifs can cooperate to increase affinity.

Two questions remain unanswered in the wake of these otherwise very informative new structures. First, given the lack of detail in the electron density for the peptide in the secondary binding site, and conflicting data from NMR studies of others (Liu et al., 2002), it is not clear if the orientation of the peptides is unique. The uncertainty is most acute for the secondary site formed by helices 2 and 3. SH3 domains can bind PPII helices in both orientations, and it would not be that surprising if FAT domains could, by analogy, bind α-helical ligands in two orientations. Second, one of the earliest findings in focal adhesion signal transduction was that FAK is phosphorylated on Tyr-925, triggering the MAP kinase cascade (Schlaepfer et al., 1994). Tyr-925 is buried by helix 1 of the FAT domain. Helix 1 is capable of domain swapping, which suggests that it is possible for the FAT domain to open up and expose Tyr-925 to the active site of FAK. However, the buried conformation is stabilized by binding to the LD motifs, since helix 1 directly participates in the major binding site. The structural observations suggest that there may be functional antagonism between phosphorylation and paxillin binding, but this remains to be seen.

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Selected Reading

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An Atomic Model of a Plant Reovirus: Rice Dwarf Virus

The structures of double-stranded RNA viruses infecting mammals and insects have been previously determined. Now the structure of the plant rice dwarf virus reveals common themes and novel features among the reoviruses.

Double-stranded (ds) RNA viruses are widely distributed pathogens and atomic models are available for the so-called core particles of human orthoreovirus (Reinisch et al., 2000), bovine orbivirus (blue tongue, BTV) (Grimes et al., 1998), and the entire yeast L-A totivirus (Naitow et al., 2002). The first atomic model of a phytoreovirus, the causative agent of rice dwarf disease, is now reported in this issue of *Structure* (Nakagawa et al., 2003). Rice dwarf virus (RDV) propagates in the insect vector that transmits it to rice as well as in the plant host (Omura and Yan, 1999). Indeed, only virus that has been through the vector is infectious for plants. Leafhoppers must feed on the plant, the virus propagated in the leafhopper and then plants are infected by deposition of the "activated" virus when the leafhopper chews on an uninfected plant. Virus purified from the plant cannot be propagated by mechanical inoculation of rice.

Like RDV, the core particle structures reported for orthoreovirus and BTV are resident in the cell, transcribe and replicate RNA within the core, and are capable of generating progeny viruses. The infectious forms of both these viruses contain an additional shell of protein that is lost during cell entry and not required for RNA replication and transcription. This outer shell probably adds some stability to the viruses and is definitely required for the cores to enter the cell. The structure of the $(\mu 1\sigma 3)_3$ isolated heterotrimer recently added insight to the entry process for reovirus (Liemann et al., 2002). RDV also contains proteins external to the P8 outer shell visual-ized in the reported structure. This gene product, called P2, has a molecular weight of 123 kDa but was removed during purification by treating the virus with CCl_4. P2, is required for oral entry into the insect vector, as feeding leafhoppers cannot be infected with virus that lacks them. Leafhoppers can, however, be infected by injection with RDV missing P2 (Omura et al., 1998).

The overall RDV structure is more similar to BTV than the orthoreovirus. RDV has a $T = 13$ outer shell formed by 260 tightly interacting trimers of the P8 protein. P8 is analogous to the VP7 protein of BTV, and, like VP7, it is composed of an internal helical domain that is formed by N- and C-terminal additions to a central sequence that forms the ubiquitous eight-stranded antiparallel β sandwich observed in ssRNA, ssDNA, and dsDNA virus capsids. The reovirus core particle lacks the $T = 13$ shell but has in its place 150 copies of the σ2 gene product that acts as a clamp to stabilize the inner protein shell formed by λ1 in reovirus. RDV has five unique interactions between the trimers of the $T = 13$ shell and the inner protein shell formed by 120 copies of the P3 gene product. Only the trimer on the icosahedral 3-fold axis has equivalent interactions between the P8 3-fold related subunits and the P3 3-fold related subunits. The P8 subunit has a concentration of positive charge on the inner surface that matches well with negative charge that is on the outer surface of the P3 subunit, suggesting that preformed P3 cores will initially bind trimers of P8 at icosahedral 3-fold axes. This nucleates two-dimensional "crystallization" of the other four categories of P8 trimer through strong side to side interactions with neighboring trimers but weak interactions with P3 at 12 unique positions on the P3 surface. The remarkable symmetry mismatch in four of the five trimer interactions demonstrate the adaptability of identical regions of the P8 protein to interact with totally different surfaces presented by P3. The P8 proteins behave as virtually rigid units at all the sites of interaction, illustrating the generic nature of the contact and suggesting that the P3 surface behaves like freshly cleaved mica when it is